First report of the mycoparasite *Verticillium epiphytum* on *Uromyces mucunae* in India

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Abstract Hyperparasitised uredinial and telial pustules of *Uromyces mucunae*, the rust pathogen of *Mucuna pruriens* were observed during screening for rust resistance. Morphological examination using light and scanning electron microscope revealed the presence of *Verticillium epiphytum*. This is the first record of *V. epiphytum* on *U. mucunae* from India.

Keywords Hyperparasite · *Uromyces mucunae* · *Verticillium epiphytum*

Rust of velvetbean (*Mucuna pruriens*) caused by *Uromyces mucunae* is a serious disease in India (Chavan and Patil 1972; Pande and Rao 1998). While screening the germplasm collection of *M. pruriens* for resistance against rust, we found white hyperparasitised uredinia and telia of *U. mucunae* (Fig. 1a).

The fungal hyperparasite was isolated on potato dextrose agar (Himedia, India) amended with streptomycin sulphate (100 ppm) and subcultured to potato carrot agar (PCA) (peeled potatoes 40 g, peeled carrots 40 g and tap water 1 l) for a week. Colonies appeared white on PCA. Three per cent KOH was used as a mounting medium. Phialides measured (10.5–) 17–48.5 (–52) × 1.5–2 (–3) μm ($n=30$), conidia measured 4–10.5 (–12) × 1.5–2 μm ($n=30$) and were falcate, one celled and present in globose heads (Fig. 1c, d). PCR was also conducted for amplification of nrITS region (ITS 1, 5.8 s and ITS 2) using primers ITS1 and 4. Sequence data have been deposited in Genbank with

accession number JN 593241. Blast searches revealed 97% similarity to an isolate designated as *Cephalosporium curtipes* var. *uredinicola* (AJ292405.1) and 96% similarity to *Lecanicillium psalliotae* (EF513027.1). Zare and Gams (2004) have also mentioned that *Cephalosporium curtipes* var. *uredinicola* cannot be clearly separated from *Verticillium epiphytum* using morphological and molecular characters (LSU, SSU and ITS). They also mentioned that *V. psalliotae* has been isolated from rust pustules but differs morphologically from *V. epiphytum*. The conidia of *V. epiphytum* have blunt ends and are held in globose heads. Further *V. epiphytum* does not produce a diffusible red pigment in agar media. Our isolates produced conidia in globose heads and no red pigment was seen diffusing in the medium. Based on these observations, the fungal hyperparasite was identified as *V. epiphytum* (Zare and Gams 2004).

Leaf bits containing parasitised pustules were sputter coated with gold (thickness 120 nm) under vacuum using a fine coat ion sputter JFC –1100 (JEOL, Tokyo, Japan). Gold-coated samples were placed on stubs in JEOL JSM 6360 (JEOL, Tokyo, Japan) for SEM. Direct penetration of the fungal spores by hyphae of the hyperparasite was observed, which confirmed it to be a mycoparasite (Fig. 1b). It appeared to be an invasive necrotroph (Gams et al. 2004). Conidial suspension of *V. epiphytum* (10^6 spores/ml) in water was sprayed on the detached leaves containing rust pustules (both uredinial and telial pustules). Water was sprayed on controls. Both uredinial and telial pustules became colonised by white colonies of *V. epiphytum* 7–8 days after inoculation. White colonies consisted of *V. epiphytum*. The hyperparasite was reisolated and confirmed to be *V. epiphytum*. The living fungal culture has been deposited in MACS Agharkar Research Institute, Pune, India (NFCCI No. 2283). The voucher specimens of rust infected leaves hyperparasitised by *V. epiphytum* have

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Fig. 1 **a** Parasitised uredinia of *Uromyces mucunae*. **b** Parasitised urediniospores of *Uromyces mucunae* (Bar=5 µm). **c** Globose conidial heads of *Verticillium epiphytum* (Bar=10 µm). **d** Conidia and globose conidial heads of *Verticillium epiphytum* (Bar=20 µm)

been deposited in MACS Agharkar Research Institute, Pune, India (AMH No. 9416) and ICAR Research Complex for NEH Region, Meghalaya, India (ICARHNEH 144).

Many fungiculous fungi have been reported as rust pathogens viz. *Eudarluca caricis* (pycnidial anamorph *Sphaerellopsis filum*), *Fusarium chlamydosporum*, *Tuberulina* sp., *Scytalidium uredinicola*, *L. dimorphum*, *Cladosporium* spp. and *V. epiphytum* (Mathivanan and Murugesan 1999, 2000; Zare and Gams 2004; Gams et al. 2004; Driessen et al. 2004). To our knowledge, this is the first report of *V. epiphytum* on *U. mucunae* from India.

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